MEMBRANE PROCESSING

OF CHEESE WHEY AND PREPARATION OF FERRIC

WHEY PROTEIN BY HEATING

by

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ABSTRACT

A concentrate containing up to 73% protein N was recovered from cheese whey by using cellulose acetate ultrafiltration membrances designed to reject solutes larger than 30,000 molecular weight by a continuous washing procedure.

Conditions necessary for increasing the ultrafiltration process for cheese whey are reported. Variables include pressure, membrane porosity, feed rate, clarification, temperature and pH. The objective was to prepare whey products with a minimum concentration of monovalent salts and maximum concentration of protein while still maintaining a high flux rate. As expected pH adjustment to 7.0 and clarification at 2000 X g for 5 min were critical in increasing flux rate. However, membrane blockage occurred and gel electrophoresis indicated that β-casein and α_s-casein were the major components responsible yet salts and lactose may also be implicated to a lesser degree.

Flux rate increased with temperature but was not affected by pressure. Results indicate that concentrating 3-4X would be practical but higher levels would be uneconomical due to the accumulation of viscous materials on the membrane.

Gel filtration showed that whey proteins are retained almost quantitatively in the concentrate while low molecular weight nitrogen containing material pass the membrane into the permeate.

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INTRODUCTION

Traditionally, whey, the by-product of cheese manu-
facture has been considered a waste product to be disposed of
as cheaply as possible. However, as anti-pollution laws have
become more rigid, processors have been forced to think of
whey in a utilization context. This in turn has led to the
recognition of the food value of whey protein in human
nutrition.

As pointed out by Mann (31) the most significant of
all for the future manufacture and utilization of whey proteins
and whey protein products involves the modern developments in
membrane separation technology.

The two related membrane processes -- reverse osmosis
(RO) and ultrafiltration (UF) -- have received considerable
attention as new tools for economically treating whey. Both
reverse osmosis and ultrafiltration are based on the ability
of polymeric membranes to discriminate between molecules on the
basis of size. Although RO and UF are often used synonymously
there are certain differences. In RO the membranes have a
much more closely knit structure which blocks the passage of
most solute molecules whilst solvent is free to pass through.
Working pressures are usually high, in the region of $4MNm^{-2}$
($600 lb/in^2$). Ultrafiltration is distinguished by the use
of membranes having relatively open structures which allow
the passage of molecules of all sizes up to the pore size of

the membrane. Pressures employed are usually low in the
region of 0.2-0.5 MNm^{-2} (30 - 70 lb/in^2).

McDonough (35) and Roualeyn (54) showed that by
controlling pore size during fabrication of the membranes
and by using different combinations of membranes and different
sequences of processing, a variety of useful products concen-
trated from whey could be obtained.

Numerous reports concerning concentration of cheese
whey by RO and UF have appeared in the literature (13, 24, 32,
34, 35, 36, 37, 38). More recently these processes have been
applied to whole milk and skim milk for the production of
various dairy products (7, 13, 15, 33, 47). Also a treatment
process based on a two-step application of UF and RO has
shown to reduce the BOD on the order of 99 percent (19, 20,
21). Yet, two major problems still remain that limit the
permeation rate achieved during processing of whey by both
RO and UF. These are concentration polarization and a gradual
fouling of the membrane during operation.

Concentration and drying are expensive because of
the low concentration (6 - 7%) of solids in the fluid whey.
Also the high proportions of lactic acid and salts in the
solids limit the utilization of dried whey in food products.
Thus, an economical process that would concentrate whey and
at the same time remove at least part of the lactic acid and
salts would greatly increase the feasibility of processing

whey for food uses. It was the purpose of this project to study the effect of varying operating parameters through the manipulation of temperature, pH, pressure, membrane characteristics and alteration of the feed (whey) itself in the hope of developing an efficient method for treating whey, making it feasible for industrial application.

LITERATURE REVIEW

Whey Concentration

In recent years the concentration of whey by RO and UF has been studied extensively (13, 24, 32, 34, 35, 36, 37, 38, 45, 46). Reid et al. (36) showed cellulose acetate to be an effective material for desalination of brackish and sea water but these membranes ahd low permeabilities. Loeb (36) improved upon them by a sophisticated film casting technique, thus making it possible to produce durable plastic films with high hydraulic permeability, coupled with the ability to block the passage of quite small solute molecules. Michaels (41) has listed the presently available membranes, their manufactures, characteristics and limitations. Included in the latter are membrane hydrolysis outside the range of pH 3.5 to 8.0 and a change in membrane properties above 35 - 40°C. These limitations have been overcome by poly-electrolyte membranes, manufactured by Amicon Corporation, which are effective over a wide range of pH and temperature. Along with the marked increase in available membranes there has been an interest in RO and UF for research and industrial use. In the dairy and food industries, however, it is of greater interest as a low cost alternative for evaporation and has an additional advantage of not requiring any heat (38).

Marshall et al. (32) applied RO to the fractionation

and concentration of whey. The whey containing 26.0% solids
was concentrated to 53.3% then cooled to $3^{\circ}C$ then agitated 24 hr
to crystallize the lactose. After centrifuging at 3000 rpm
for 24 hr three fractions were obtained -- a precipitate,
infranate and supernatant comprising respectively 56.6, 19.7
and 23.7% of the total weight. The precipitate was largely
lactose while 75% of the infranate solids contained protein.
However, they indicated two problems that limited the per-
meation rate achieved during processing of whey by this
method -- concentration polarization and gradual fouling of
the membrane during operation.

McDonough et al. (36) showed that a fourfold
concentration was possible but higher levels would be uneco-
nomical due to clogging problems from an accumulation of
viscous materials and insoluble solids. Glover (15) without
seeking the optimum conditions for the process showed that
protein forms a gel which adheres to the membrane and in
effect adds another filtering layer. Lim et al. (28) showed
that the major cause of blockage was due to casein but
α-lactalbumin and β-lactoglobulin were also present.

Flux decline has been attributed to such factors
as accumulation of fouling material on the membrane, concen-
tration polarization, decrease in driving force with increase
in concentration and compaction of the membrane (32, 36, 48,
50).

Peri et al. (50) working with whey and a simulated milk showed that the permeability and selectivity of the membranes were attributed to the differences in the relative importance of osmosis and pore flow. They showed that by using a clarified whey with increase in pressure that there was a subsequent increase in retention. Blockage again was found to be due to protein and other high molecular weight substances. However, the effect was readily reversible by washing with water which restored the original permeability and selectivity of the membrane.

Only by seeking optimum conditions for RO and UF would their industrial application be feasible. Forbes (14) showed that by the selection of the correct operating temperature, pH and feed velocity that the protein layer responsible for the clogging of the membrane is totally redispersible so that equilibrium and steady output are obtained. Peri and Dunkley (48) reported the influence of composition of the feed on the performance of cellulose acetate reverse osmosis membranes using varied operating pressures but constant flow-conditions. They found that fouling decreased permeation rate but its influence on retention was variable and depended principally on the feed, the solute and the available driving force. In a subsequent paper by the same workers (49) they found that by modifying the flow condition to increase turbulence, improved performance of RO membranes.

Roualeyn et al. (54) described methods to produce a variety of useful product concentrates from raw whey using different combinations of UF and Ro membranes and different sequences of processing operations. They showed that the protein: lactose ratio in the concentrate was a function of the permeability and selectivity characteristics of the membrane, as well as the system design and operating conditions. Ratios ranged from 1:8 raw whey through 3:5 to 2:1 or higher. McDonough et al. (34) using Calgin-Havens type 215 membranes and optimum conditions have shown rejection values of 97.4% for protein, 4.8% for ash, 3.9% for lactic acid and 13.4% for lactose, the latter being slightly higher than desired.

Recently Horton et al. (19) have set up the first commercial scale UF/RO plant for fractionating cottage cheese whey into protein and lactose concentrates capable of handling 300,000 lb of cottage cheese whey oer day using sanitary clean-in-place equipment.

Concentration and Fractionation of Milk and Skimmilk by RO and UF

There are two main reasons for concentrating milk: first to facilitate the transport of liquid milk and second to reduce the volumes of liquid involved in cheese making. Glover (15) was able to concentrate milk 2X without any flavor changes while retaining all the main constituents of the milk. Although fouling was inevitable, this could be overcome by a two-step process. Ultrafiltration could be

used first to concentrate the fat and protein, followed by RO
to concentrate the remainder of the components, after which
the products of both processes would be recombined to produce
a concentrated milk.

Ultrafiltration of skim milk has been made in a
concentration ratio of 6:1 by Maubois and Mocquot (33) and
the concentration was used for the production of Camembert
cheese whose organoleptic qualities were found to be equivalent
to the cheese prepared according to the conventional procedure.
In addition to the increase in yield, reduction in pollution
due to the whey and certain technological advantages are
claimed; such as improvements in factory design, less hand
work and less weight fluctuation from one cheese to another.

Fenton-May et al. (13) were able to concentrate
skim milk to 22% total solids by RO or to fractionate it by
UF to produce liquid concentrates with 50 to 80% protein
(dry basis). Proteinaceous deposits on the membranes resulted
in a measurable resistance to transport and consequently the
permeate flux. They suggested therefore that systems be
designed to operate with high feed velocities to minimize
this boundary layer thickness.

Bundgaard et al. (7) succeeded in producing by
ultrafiltration a skim milk concentrate of such a composition
that it could be used for the production of fresh cheese,
white mould cheese and a semi-hard cheese. Moreover, by a

RO treatment of the UF permeate the concentrates would be made suitable for calf feed as well as enabling utilization of the lactose for yogurt production.

More recently Peri et al. (47), by combining ultra-filtration and washing, were able to obtain concentrations of skim milk proteins up to values of 80% on a dry weight basis.

Application of RO and UF to other areas

Applications of RO to foods can be classified into three areas: concentation, purification or fractionation and waste disposal (26, 40). As proposed by Morgan et al. (42) these methods can be used for the concentration of liquid food products economically without phase change or the application of heat. Lowe et al. (30) using a Wurstack RO arrangement, developed at the Western Utilization Research Division successfully concentrated egg white 2.5 times. In addition other products processed with varying degrees of success include: lemon, orange, tomato, whole milk, grape, coffee and apple. A 30% solids egg white concentrate with excellent functional properties equal to that of fresh egg white was also produced (29).

Apple and orange juice were concentrated fourfold by RO (34). In spite of the loss of some water soluble aroma compounds the flavor of the concentrate was judged to be excellent. In commercial evaporation of orange juice, all of the water-soluble aroma compounds are completely

stripped off. To compensate for this,commercial practice is to add peel oil and to over-concentrate. Therefore, ultra-filtration overcomes these problems.

Although the cost is slightly more expensive than thermal evaporation equipment, 75% of the water was removed from maple sap by RO (59), with about 1 part in two thousand sap solids lost in the by-product water.

Experiments in applying UF to enzyme recovery have been encouraging. It has been possible to concentrate up to sevenfold or more at flux rates of 10 - 20 gal/day ft^2 with enzyme retentions of 90 - 95% while still retaining 95 - 98% of the enzyme activity (9).

UF can also be applied to the concentration of viruses and this has a wide potential implication for the manufacture of sera and vaccines. Concentration ratios of 18 - 19 were achieved in various media with little measured loss of activity (9).

Membrane Separation Process for the Abatement of Pollution

The establishment of new standards and requirements for effluent quality water has accentuated the need for new and improved routes for waste treatment (56). Moreover, anti-pollution concern is forcing processors to look towards utilization rather than disposal (35).

UF and RO treatments eliminates the need for high capacity settling equipment and use of coagulants and

flocculants as settling aids and clarifiers in waste treatment operations (41).

RO equipment and operating procedures are relatively expensive for processing spent liquors from the pulp and paper industry in amounts of several million gallons per day compared to conventional methods (57). Yet, the treatment (through high-flux, low pressure membranes) of primary and secondary sewage effluents yields crystal clear, microorganism-free filtrates from which virtually all biologically active taste, color and odor-producing contaminants have been removed (41). Moreover, effluents are of a low BOD suitable for discharge without pollution and the concentrate can be readily desiccated and incinerated or processed economically for by-product recovery. Recoveries of excellent quality water, well in excess of 80%, have been obtained. Thus capabilities of substantially extending the recycling of water within pulp mill systems are possible (58).

A whey treatment process which uses a two-step application of UF for the first step and RO for the second has shown to be able to reduce the BOD in the order of 99% (19, 20, 21). Also the BOD of raw whey has been reduced from 50,000 - 60,000 ppm to 1000 ppm after UF treatment (9). At present the Crowleys Milk Company Inc. is able to process 300,000 lbs of cottage cheese whey per day (19).

Besik et al. (4) pointed out that a 39.3% return on capital is possible by processing whey through a combination

of RO with spray drying and at the same time solving the
unpleasant pollution problem.

METHODS AND MATERIALS

Ultrafiltration

Ultrafiltration was done on a small laboratory-scale apparatus (Model 52, Amicon Corporation, Lexington, Massachusetts, U.S.A.) illustrated in plates I and II, having a maximum internal volume of 65 ml. It consisted of a cylindrical container 130 mm high, 43 mm internal diameter, with a horizontal porous support disc near the bottom supporting a cellulose acetate membrane 1250 mm^2 in area. Immediately above the membrane was a magnetic stirrer. The membranes used were in the form of flat sheets, and designated as types UM05, PM10, PM30, XM100 having minimal cutoffs at molecular weights 500, 10,000, 30,000 and 100,000 respectively. These values may have been only approximate since it was found that another membrane having the same molecular cutoff point had different permeation rates. Therefore, in order to standardize the membrane, the permeation rate of distilled water was measured over a half hour period. Pressure was applied from an air cylinder at 0.4 MNm^{-2} (60 lb/in^2) and the experiments were carried out at temperatures between 25 and 45°C. Turbulence was maintained at a constant speed in the UF cell by means of the magnetic stirrer. The filtrate was taken out through a tube just below the porous support disc and collected in a graduated cylinder.

In order to supply liquid to the ultrafiltration cell when the volume of liquid to be processed exceeded the
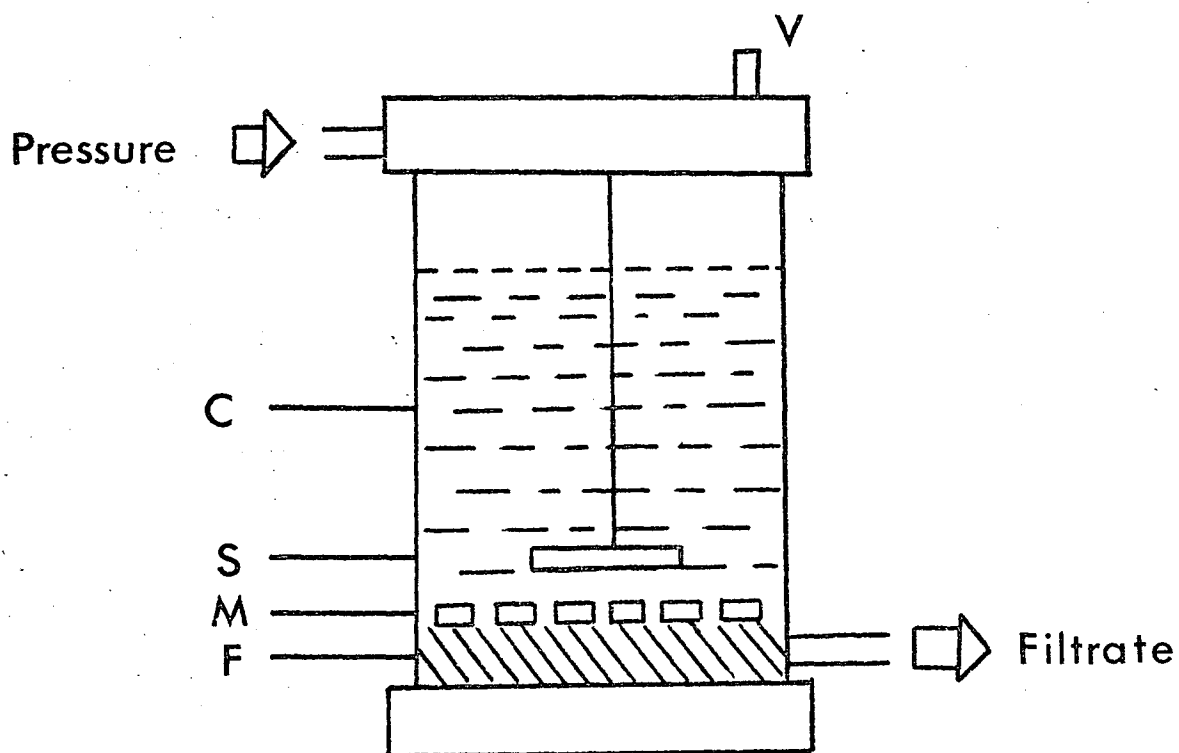
Plate I. Ultrafiltration apparatus -- laboratory scale.
C, concentrate; F, filtrate; M, membrane
mounted on porous plastic supporting disc;
S, magnetic stirrer; V, filling tube and
relief valve.
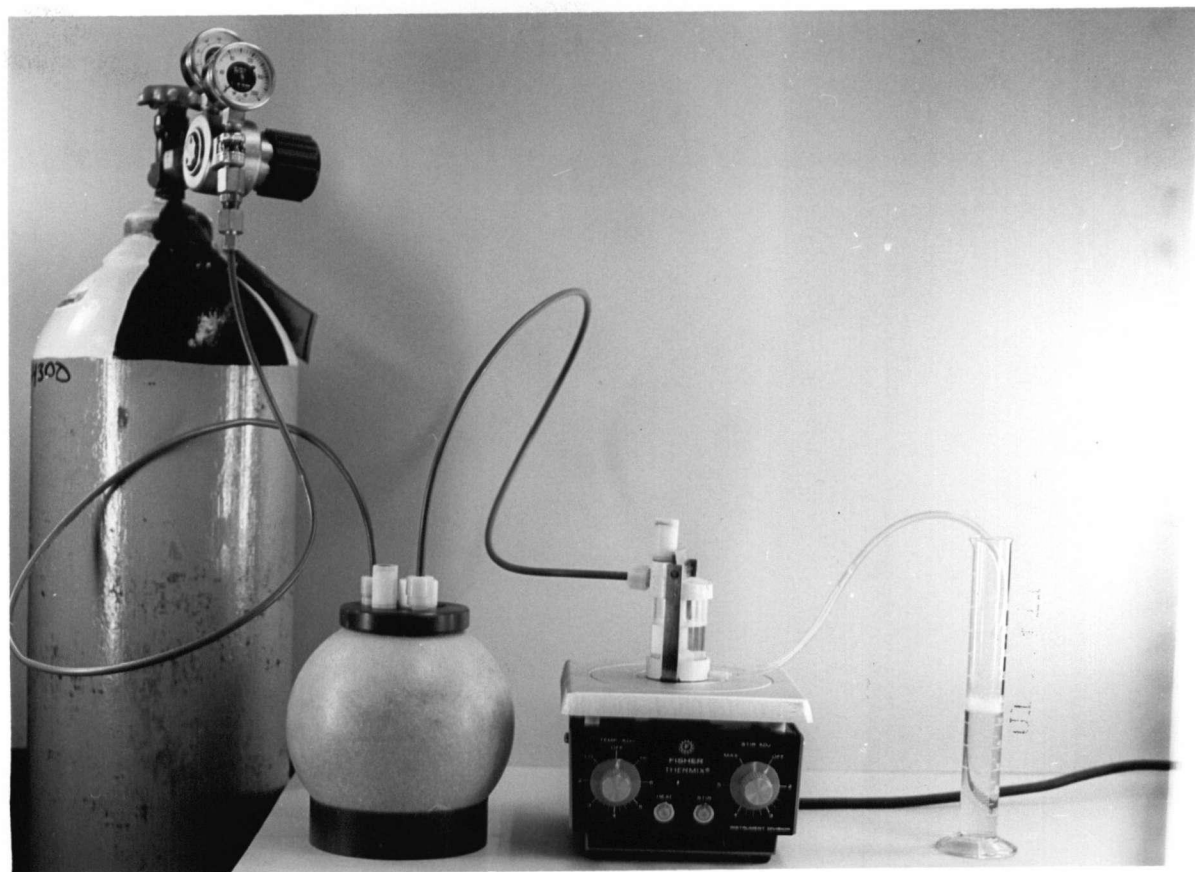
Plate II.　　　Ultrafiltration Assembly

    1.　Air cylinder

    2.　Fiberglass reservoir

    3.　Ultrafiltration chamber with membrane

    4.　Magnetic stirrer

    5.　Graduated collecting cylinder

cell capacity, the ultrafiltration cell was connected to a fiberglass liquid reservoir (Model RG-3 Amicon Corporation, Lexington, Massachusetts, U.S.A.), illustrated in plate II having a maximum internal volume of 2750 ml. Membranes were conditioned before using by rinsing in a beaker of distilled water for at least one hour with one change to remove the glycerine added.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis was performed according to Aschaffenburg's method (2) with modifications. The gel was prepared as follows: 10.5 g acrylamide, 0.52 gm N,N-methylenebis acrylamide and 40 g urea were dissolved in 0.175M Tris-glycine buffer pH 9.1 and made up to a final volume of 150 ml. This solution was filtered through No.1 filter paper into a vacuum flask. After deaeration 0.45 ml mercaptoethanol, 1.25 ml TMED (30% N, N, N, N, - tetra-methylene ethylenediamine in 95% ethanol) and 1.25 ml of 10% ammonium persulfate were added to the solution in that order, mixed gently, poured immediately into a mold, covered with a plexiglass plate and weighed down to exclude the air. The gel was allowed to stand 30 - 60 minutes to permit complete polymerization. A horizontal electrophoresis apparatus with two troughs at either end of the gel was used with a sodium chloride solution 0.1 M in the outer and 0.175 M Tris-glycine buffer in the inner. The two buffers were connected with cheesecloth bridges. The gel was placed

on the electrophoresis apparatus and covered with saran wrap
extending over the cheesecloth bridges to avoid drying. The
gel was equilibrated for 20 hr at $4^{\circ}C$ by running a current
through it. The amperage was maintained at 20 mA by a power
supply.

Preparation of Sample

A 2% solution of each of the following samples was
prepared in 0.175 M Tris-glycine buffer:

1. β-lactoglobulin
2. K-casein
3. whey pasteurized at $72^{\circ}C$ for 10 min after adjusting
    to pH 7.0
4. acid casein
5. the supernatant after centrifuging No. 3 at 1,500
    X g for 10 min at $0^{\circ}C$.
6. the sediment of No. 5
7. ferric whey protein containing 224 μg/ml whey.

To each sample except No. 7 was added one drop of mercapto-
ethanol. Strips of Whatman No.3 MM filter paper 1.5 cm X
0.2 cm wide were saturated with each sample and blotted on a
clean tissue. With the aid of a razor blade they were then
inserted into the slot on the equilibrated gel plate. Electro-
phoresis was carried out under the same conditions as the
equilibration of the gel. After migration was completed the
gel was removed from the mold and stained for 10 min in a 1%
amido black dye solution in 10% acetic acid. Destaining was
accomplished by continuously washing the gel in 5% acetic
acid until the protein bands appeared blue against a clear
background.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was done using the ACI
Cassette Electrophoresis Cell and Power Supply and the ACI
Agarose Universal Electrophoresis Film (Analytical Chemists
Inc., Palo Alto, California, U.S.A.) illustrated in Plate III.
All procedures were carried out at 4°C. The electrophoresis
film was removed from its plastic support and placed in the
stir dish (plate III) and conditioned for one hour in 200 ml
of 4M urea made up in Tris-glycine buffer pH 9.1. After
conditioning, the film was removed and dryed so that no buffer
remained in the sample slots.

Preparation of Sample

A 3% solution of each of the following was prepared
in Tris-glycine buffer pH 9.1 plus one drop of mercaptoethanol.

1. β-lactoglobulin
2. K-casein
3. acid casein
4. the sediment of No.6
5. the supernatant after centrifuging No.6 at 1,500
   X g for 10 min at 0°C.
6. whey pasteurized at 72°C for 10 min after adjusting
   to pH 7.0.

Samples were applied to the film slots by using the micro-
liter dispensor with disposable sample tips. Electrophoresis
was then carried out by placing the film in the cassette
cover, which was fitted into position on the cassette holder
containing about 200 ml of sodium barbital buffer, pH 8.6,
0.5 M with 0.035% EDTA. The cassette assembly was then
attached to the power supply and run for one hour. Following
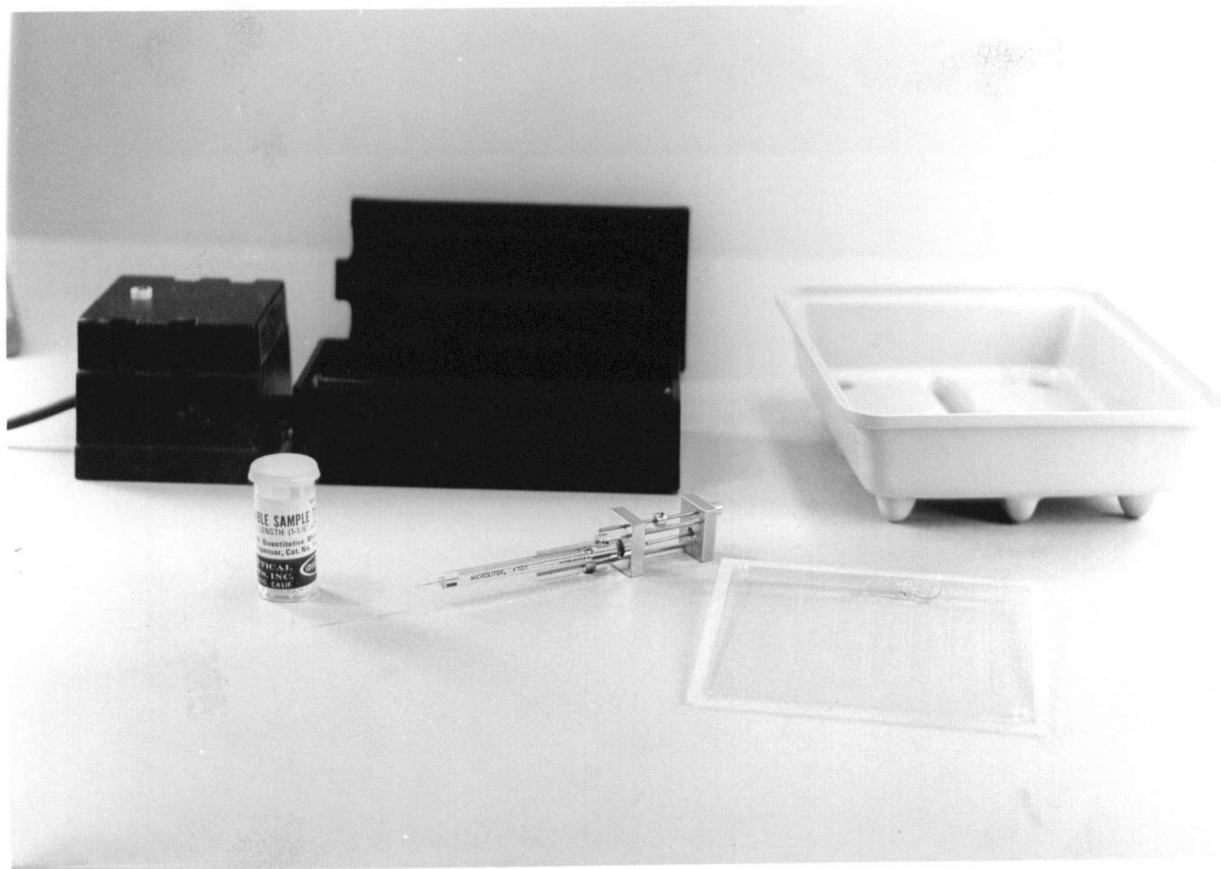
Plate III.   ACI Agarose Film Cassette System

1. Power supply

2. Cassette

3. Cassette cover

4. Agarose gel plate

5. Quantitative microliter sample dispensor

6. Disposable sample tips

7. Stir-stain dish

electrophoresis the proteins were simultaneously fixed and
stained by immersing the film in approximately 200 ml of 0.2
g % Amido Black in 5% acetic acid for 15 min. The excess
Amido Black was removed by placing the film in a stain dish
of 5% acetic acid for 30 seconds with agitation. The film
was then dried at a temperature between 75 - 85°C in a
convection oven (plate IV). Destaining was accomplished by
placing the film in 5% acetic acid until the majority of
the stain was cleared. This required approximately one
minute. The remaining background stain was removed by placing
the film in a dish containing clear 5% acetic acid for one
minute. The film was then dried in the oven as before. The
entire procedure including conditioning of the film takes
approximately three hours.

Acid Whey

Cottage cheese whey was obtained from a local dairy
(Fraser Valley Milk Producers Association, Vancouver, Canada).
The whey was pasteurized at 72°C for 10 min, adjusted to pH
7.0 and centrifuged at 1,500 X g for 5 min at 0°C using a
Sorvall RC2-B centrifuge. The supernatant was then used for
the ultrafiltration experiments. Whey samples were held at
4°C for no longer than 2 days.

Preparation of Ferric Whey Protein

As described in Part II.

Solubility

A suspension of 0.2 g freeze dryed whey product
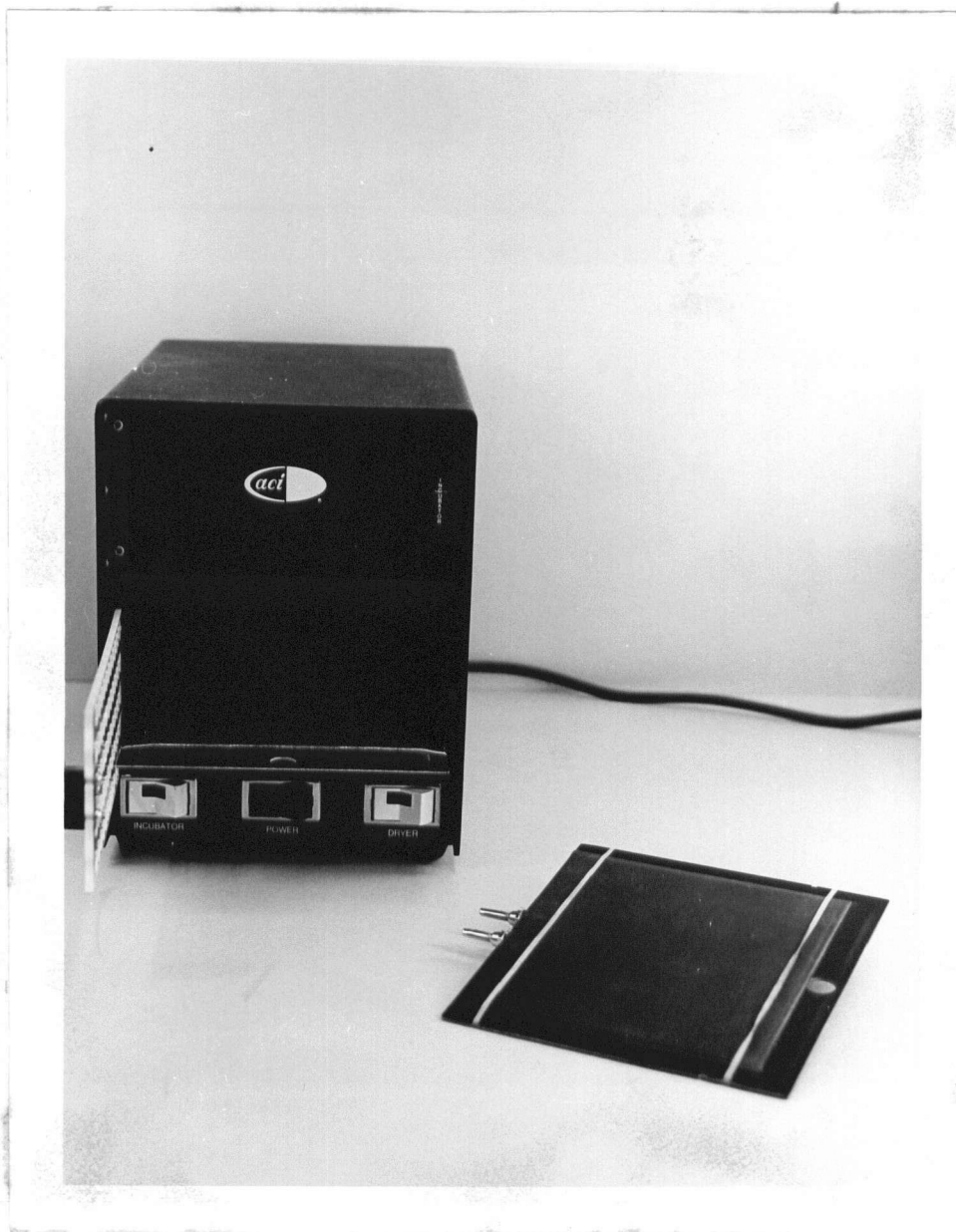
Plate IV.

1. Convection oven

2. Heated agarose plate support

in 20 ml of water at 25°C was blended in a Lourdes Model

MM - 1A multimixer (Lourdes Instrument Corporation, New York)

at 18,000 rpm. Protein in the supernatant after centrifuging

at 2,000 X g for 19 min was determined. The solubility was

expressed as a percentage of total protein in the original

sample.

Nitrogen Determinations

Nitrogen was determined according to the dye binding

procedure (12) for routine analysis. A micro-kjeldahl method

(3) was used as a standard method. Total protein was deter-

mined by the factor 6.38 X total nitrogen.

Lactose

Lactose was analyzed by an anthrone method (57).

Total Solids

Total solids were determined according to the

Official AOAC method (3).

Ash

Ash analysis was done according to the Official

AOAC method (3).

Iron

A colorimetric method using ferrozine was applied

after deproteinization (8) or Jarrel-Ash Atomic Absorption

Spectrophotometer 82 - 800 was used after dry ashing.

Comparable.
Agreeable results were obtained between both methods.

Calcium and Magnesium

A back titration using calcium chloride and a

calcium indicator (44) was used directly or Jarrel-Ash Atomic Absorption Spectrophotometer 82 - 800 was used after dry ashing.

Phosphorous

Phosphorous was determined spectrophotometrically after wet ashing using sulphuric acid (43).

Biological Oxidation Demand

BOD was analyzed using the Official Standard Method (56) with a 5 day incubation period of 20°C. All whey samples were fresh and stored at 4°C for no longer than 2 days. Samples including acid whey, ultrafiltered concentrates and permeates were diluted 100 times and 5 ml of this dilution covering a BOD range of 12,000 - 36,000 ppm was used for analysis.

Gel Filtration

Gel filtration was carried out in Sephadex G-50 in a column fitted with flow adaptors and operated in an upward flow manner. The eluting buffer was 0.05 M ammonium acetate pH 4.5 and the sample was applied by means of a pipette (52).

Membrane Regeneration

Membrane regeneration was accomplished by back-washing first with approximately 60 ml of NaOH pH 11.5 followed by the same amount of 0.5 N HCl. Regeneration was repeated if the permeation rate of distilled water at 25°C

over a half hour period did not approach the same rate as that of a new membrane under the same conditions. Membranes were replaced if the permeation rates of distilled water after regeneration were low.

Temperature

The effect of temperature on permeate flux was studied by placing the filtration apparatus in a controlled circulating water bath. Temperature ranged from 25 - 45 ± 0.5°C. The process was not started until the desired temperature of the whey was reached.

RESULTS

Rates

Table I gives the rates of the ultrafiltration process at 25°C. Average permeate fluxes for whey ranged from 10 ml per m^2 per hr for UM 05 membrances to 34 ml per m^2 hr for XM 100 membranes. Generally the permeate flux decreased with time as the process continued. However, there was an increase in permeate flux with temperature increase (Figure 7).

Ferric whey protein was prepared according to the method outlined in Part II, omitting the preconcentration step. Although ultrafiltration of the supernatant was 83% lower in protein it did not substantially increase permeate flux (Table I).

Membrane Selection

The first part of the work undertaken was concerned with selection of a membrane. Table II gives the chemical analysis of the fractions and the levels of retention of the various membranes. These results were obtained from unwashed concentrates and permeates. It can be seen that with the PM 30 membrane 94% of the protein and 53% of the lactose remained in the concentrate. Various protein : lactose ratios were obtained with different membranes. Washing of the protein concentrate removed residual lactose and made it possible to increase the protein concentration to greater than 70% (Table III).

TABLE I

Rates of the ultrafiltration process

using different membranes

at $25^{\circ}C$

Memb. type	Sample	Orig. vol.	Final Vol.	Time hr.	Conc. factor by vol.	Temp. $^{\circ}C$	Permeate flux hr.
		ml	ml		ml		ml/hr
UMO5	whey	60	50	1.0	1.20	25	10
PM10	whey	60	28	1.0	2.14	25	32
PM30	whey	60	29	1.0	2.06	25	31
XM100	whey	60	26	1.0	2.30	25	34
XM100[1]	Fe whey	60	27	1.0	2.22	25	33

[1] Ferric whey protein containing 224 mg Fe/ml.

Whey + Fe → $90^{\circ}C$ for 10" → centrifuge at 3000 rpm for

10" → adjusts pH of supernatant to 7.0 → centrifuge

same speed → UF supernatant.

TABLE II

Effect of membrane type on retention of

selected fractions in the ultrafiltration

of whey

| Memb. type | Sample | RETENTION % | | | |
		Protein	Lactose	Total solids	Ash
UMO5	whey	92.71	89.35	90.22	61
PM10	whey	93.81	51.60	55.42	70
PM30	whey	94.44	52.61	58.86	70
XM100	whey	89.80	47.87	50.48	52
XM100	Fe whey	82.39	46.81	53.26	56

(Membrane retention stated as amount retained in the concentrate expressed as percentage of the component in the original sample).

TABLE   III

Analysis of whey protein concentrate and crude

soluble lactose (freeze dried)

Product	Moisture %	Total solids %	Protein %	Lactose %	Ash %	Ca %	Mg %	Phosphorous %
Cottage cheese whey protein	3.45	.96	72.8	18.0	.69	.059	.023	1.3
1st lactose	4.55	.95	6.5	86	.91	.061	.026	.56
2nd lactose	4.04	.96	6.5	83	.13	.073	.030	.70

Whey concentrated 3X then washed continuously with 166 ml H_2O.

Protein % N X 6.38.

.All subsequent experiments were done using a PM 30 membrane with molecular cutoff at .30,000.

Decline in Permeate Flux

Figure 1 shows the decline in permeate flux during the ultrafiltration of pasteurized cheese whey pH 4.6 at 25°C using a PM 30 membrane at an operating pressure of 60 psi. From the graph it can be seen that after 25 hours the membrane is so clogged that the permeate flux is essentially zero.

Relationship Between Flux and pH

The effect of pH on membrane flux is rather dramatic as illustrated in Figure 2. There is no substantial difference in permeate rate between centrifuged and uncentrifuged samples in the pH range 3 - 5. However, the difference is large in the pH range 5 - 8. Maximum flux was obtained with the centrifugal samples at pH 7.0.

Effect of Centrifuging

From the results obtained in Figure 2 it can be seen that centrifuging has a great effect on increasing flux rate. Protein determination done on the supernatants after centrifuging (Figure 3) show that maximum flux is associated with the minimum concentration of protein. Centrifuging at pH 6 - 8 produced a slight precipitate which was assumed to be partly responsible for the decrease in flux.

Polyacrylamide Gel Electrophoresis

Polyacrylamide and Agarose gel electrophoresis were done to determine what proteins were responsible for
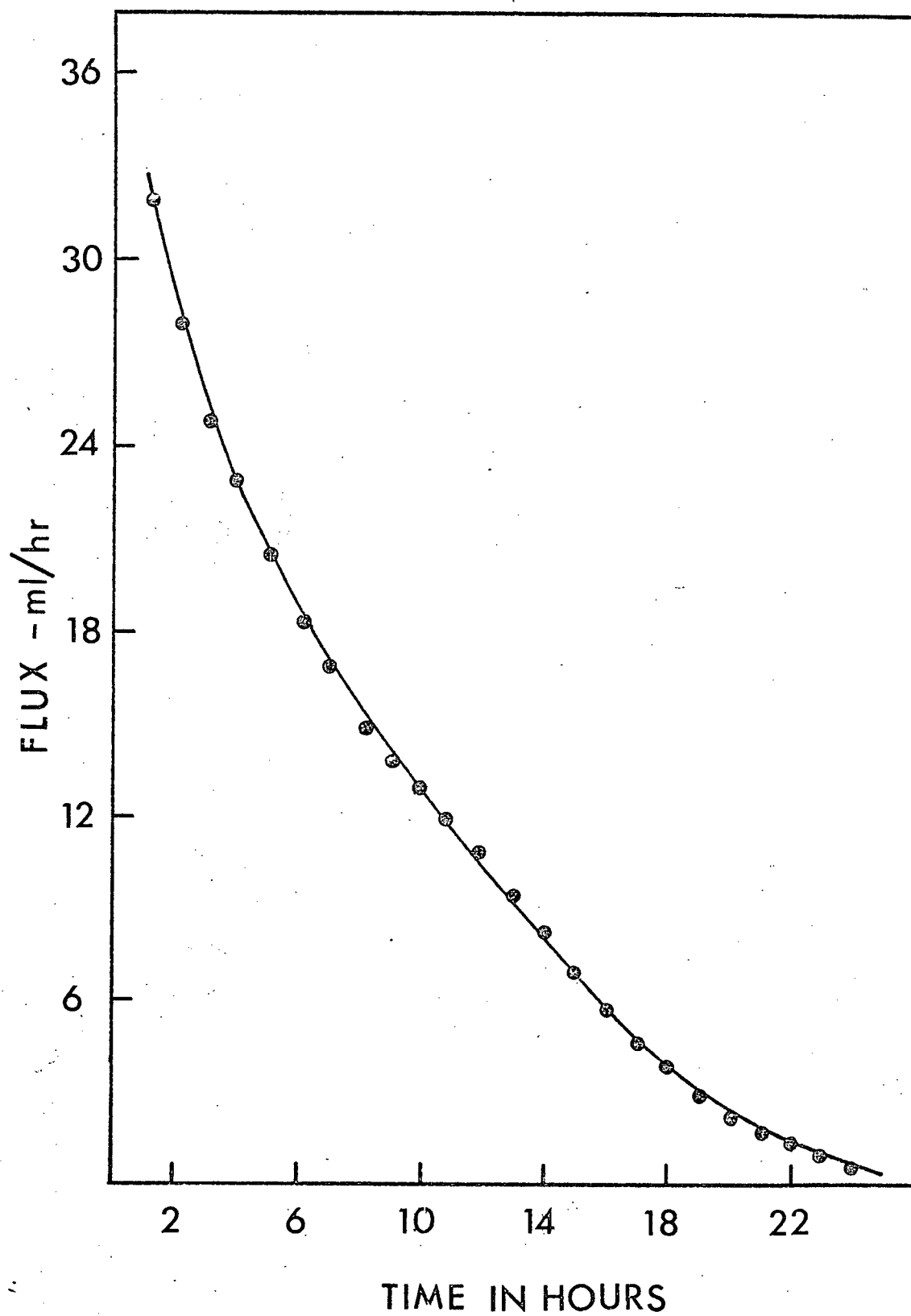
Figure 1. Decline in permeate flux during ultrafiltration of whey at pH 4.6.
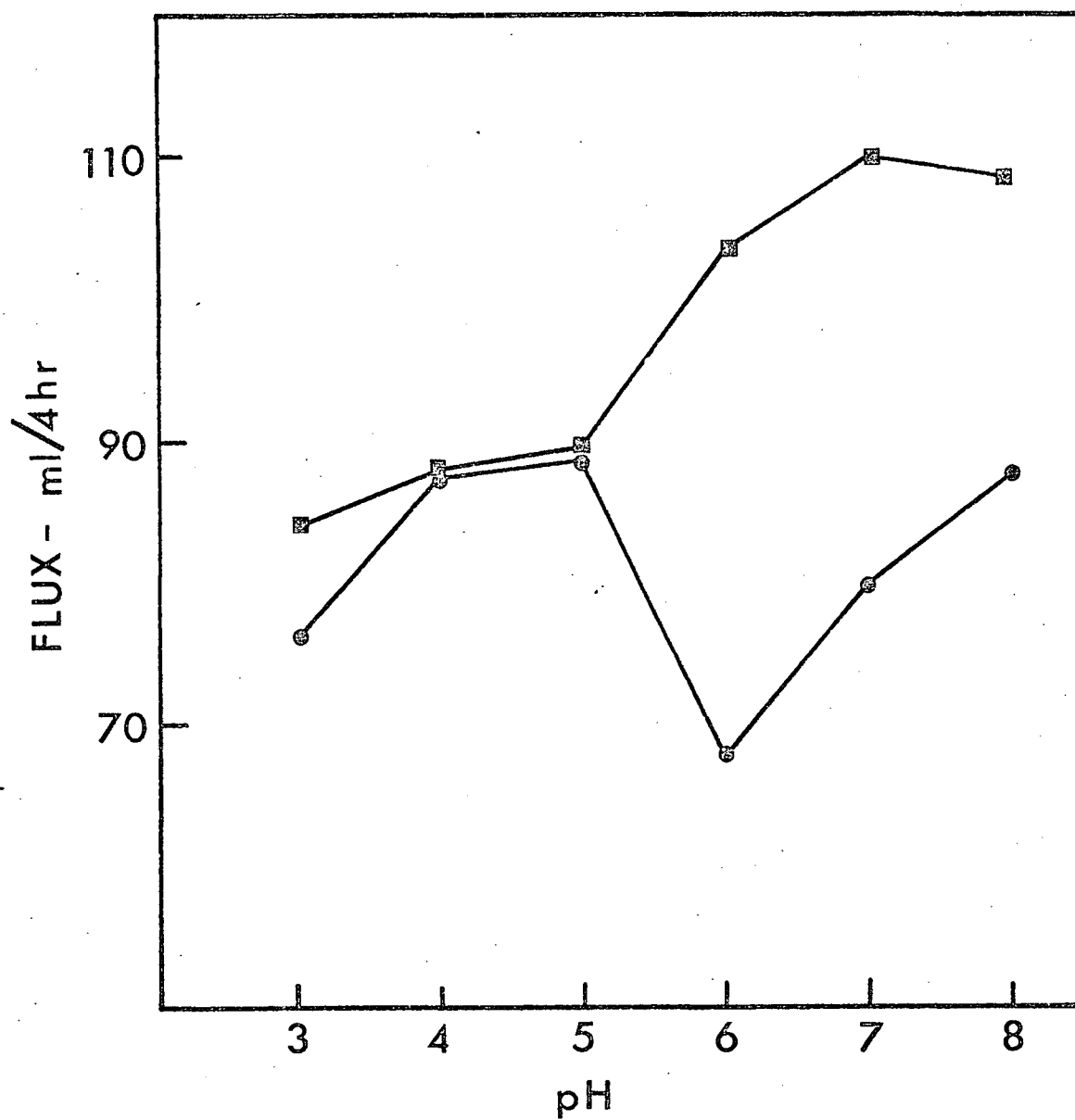
Figure 2.   Relationship between permeate flux and pH of the whey

⊚————⊚   No centrifugation after pH adjustment

▓————▓   Centrifuged at 1500 X g for 5 min
              after pH adjustment.
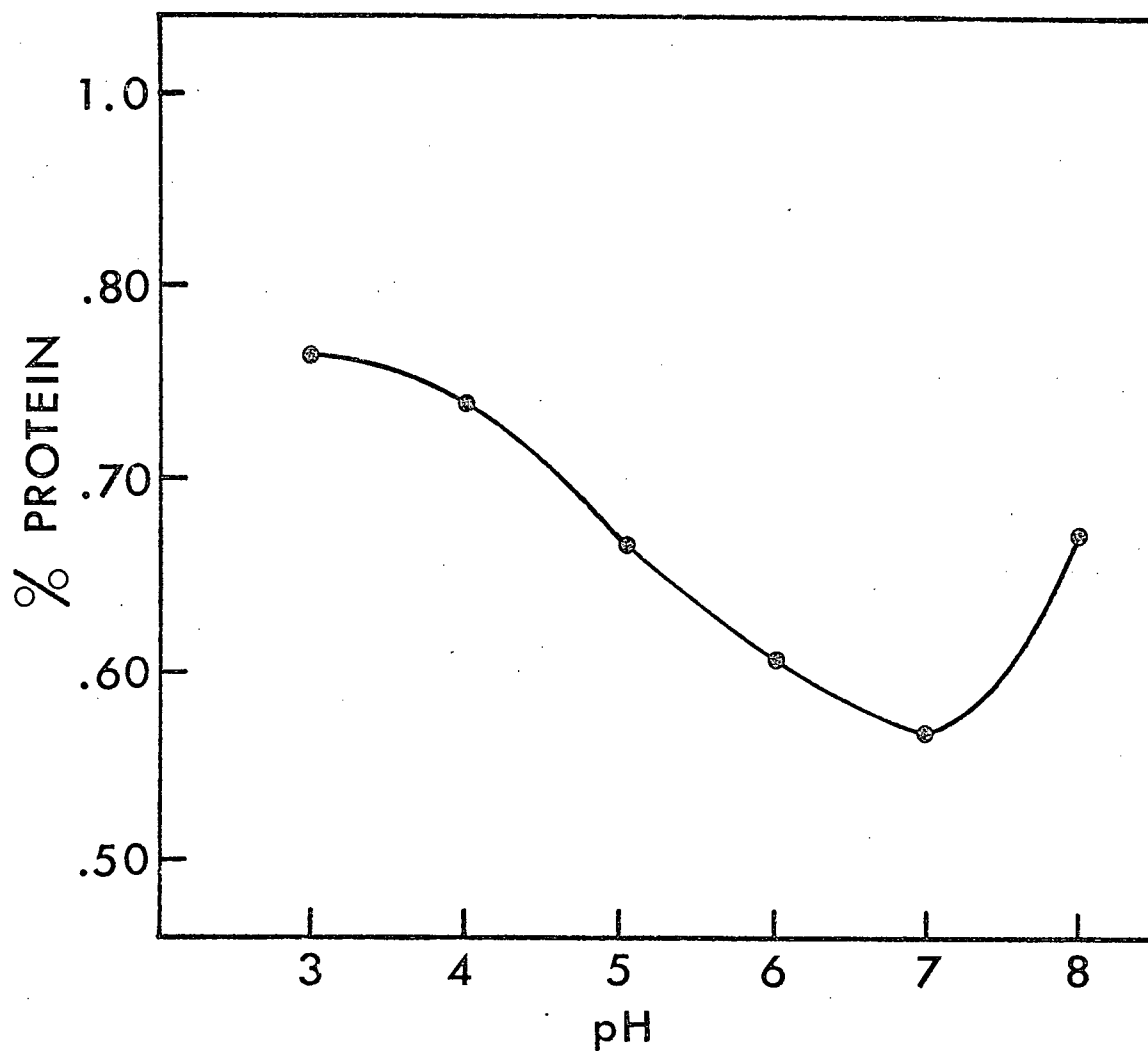
Figure 3. Effect of centrifugation on protein precipitation

the decrease in flux (Figure 4). From the results it can be
seen that β-casein and α_s-casein are responsible to a certain
degree in the clogging of the membrane. Also, the α_S-casein
band disappears after centrifuging (Figure 4 (5)) while it
is still present in the uncentrifuged sample. Agarose gel
electrophoresis did not give good separation (Figure 5). It
was used because of its speed and simplicity and answered
question which would otherwise have to be obtained through
polyacrylamide gel electrophoresis.

Gel Permeation

  Determination of the relation between total nitrogen
and true protein retention was accomplished by performing a
series of gel permeation analyses, typical data from which
are illustrated in Figure 6. Two curves are shown: one for
whey and one for permeate. It can be seen that the lower
molecular weight nitrogen-containing material such as free
amino acids, peptides and urea pass through the membrane into
the permeate while the proteins are retained almost
quantitatively in the concentrate.

Effect of Washing

  Washing the concentrate removes residual lactose and
thereby increases the concentration of proteins up to values of
70% on a dry weight basis (Table III). The calculation adopted
was that of Stahl (55). By increasing the number of washings
it was possible to decrease the amount of water needed
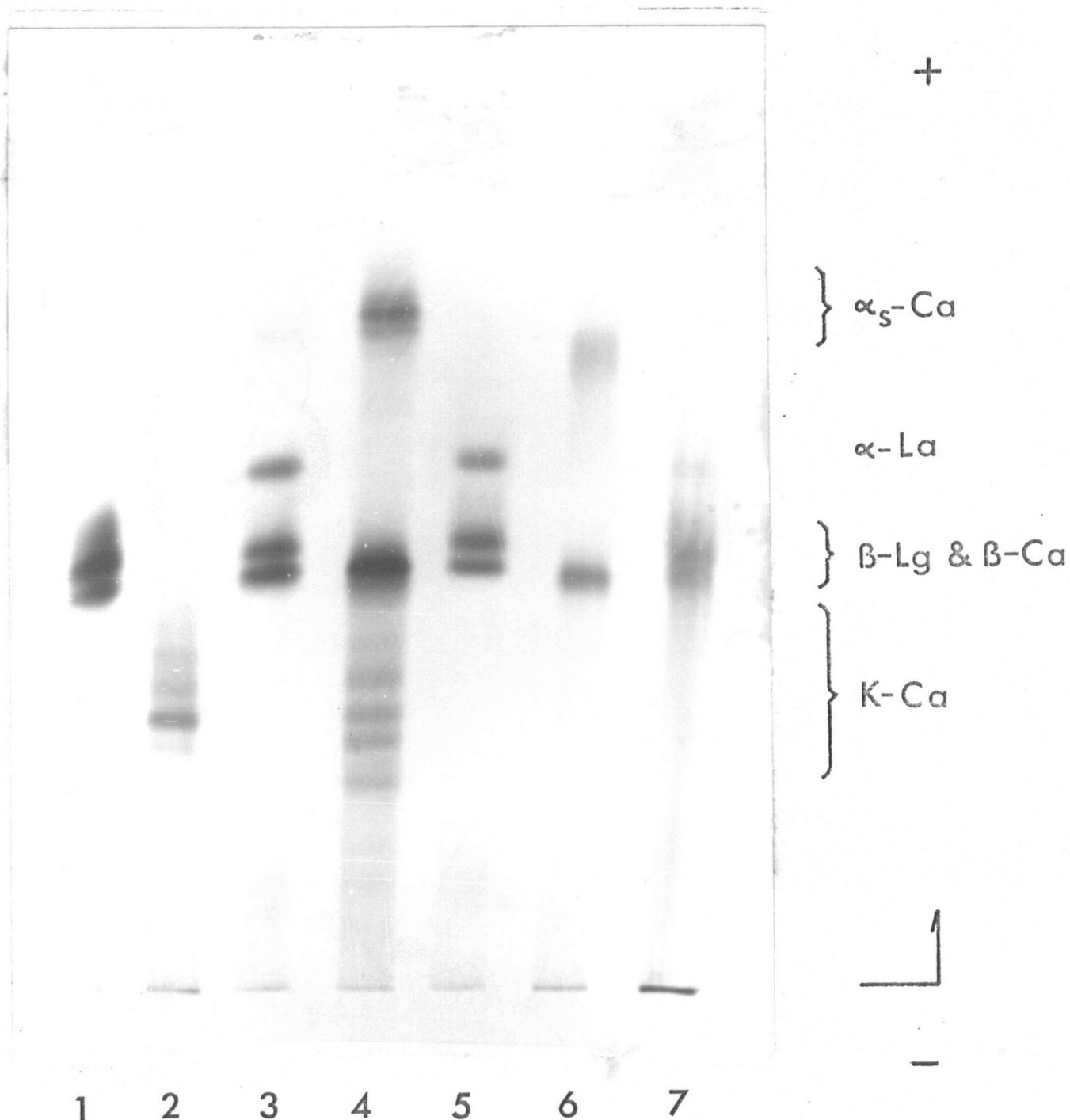
Figure 4.   Polyacrylamide gel electrophoresis of whey
             proteins. 1. β-lactoglobulin; 2. K-casein;
             3. whey pasteurized at 72 C for 10 min after
             adjusting the pH 7.0; 4. acid casein;
             5. the supernatant after centrifuging No.3
             at 1,500 X g for 10 min at 0 C; 6. the
             sediment of No. 5; 7. ferric whey protein.
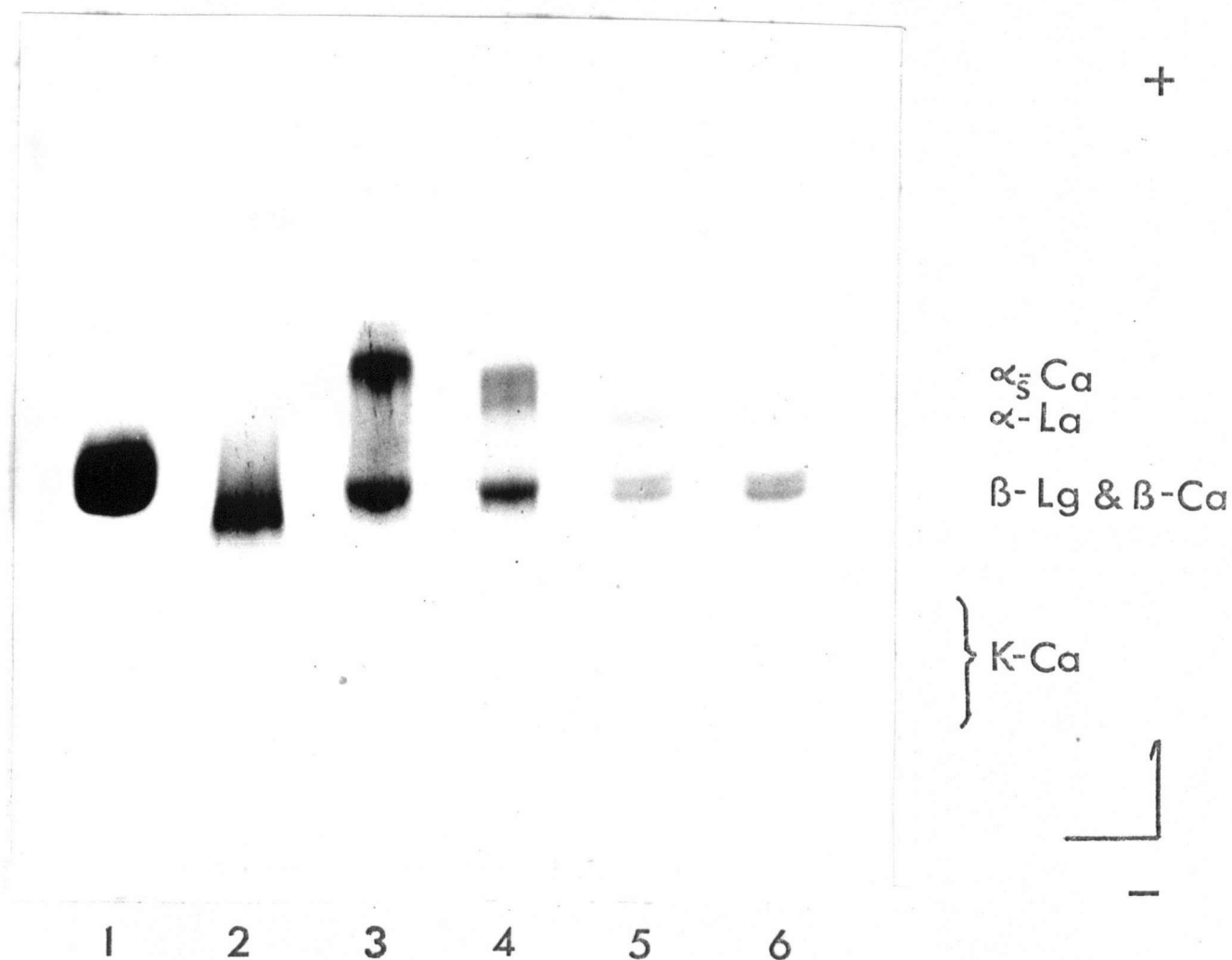
Figure 5. Agarose gel electrophoresis of whey proteins
1. β-lactoglobulin; 2. K-casein; 3. acid
casein; 4. the sediment of No.6; 5. the
supernatant after centrifuging No.6 at 1,500
X g for 10 min at 0 C; 6. whey pasteurized
at 72 C for 10 min after adjusting to pH 7.0.
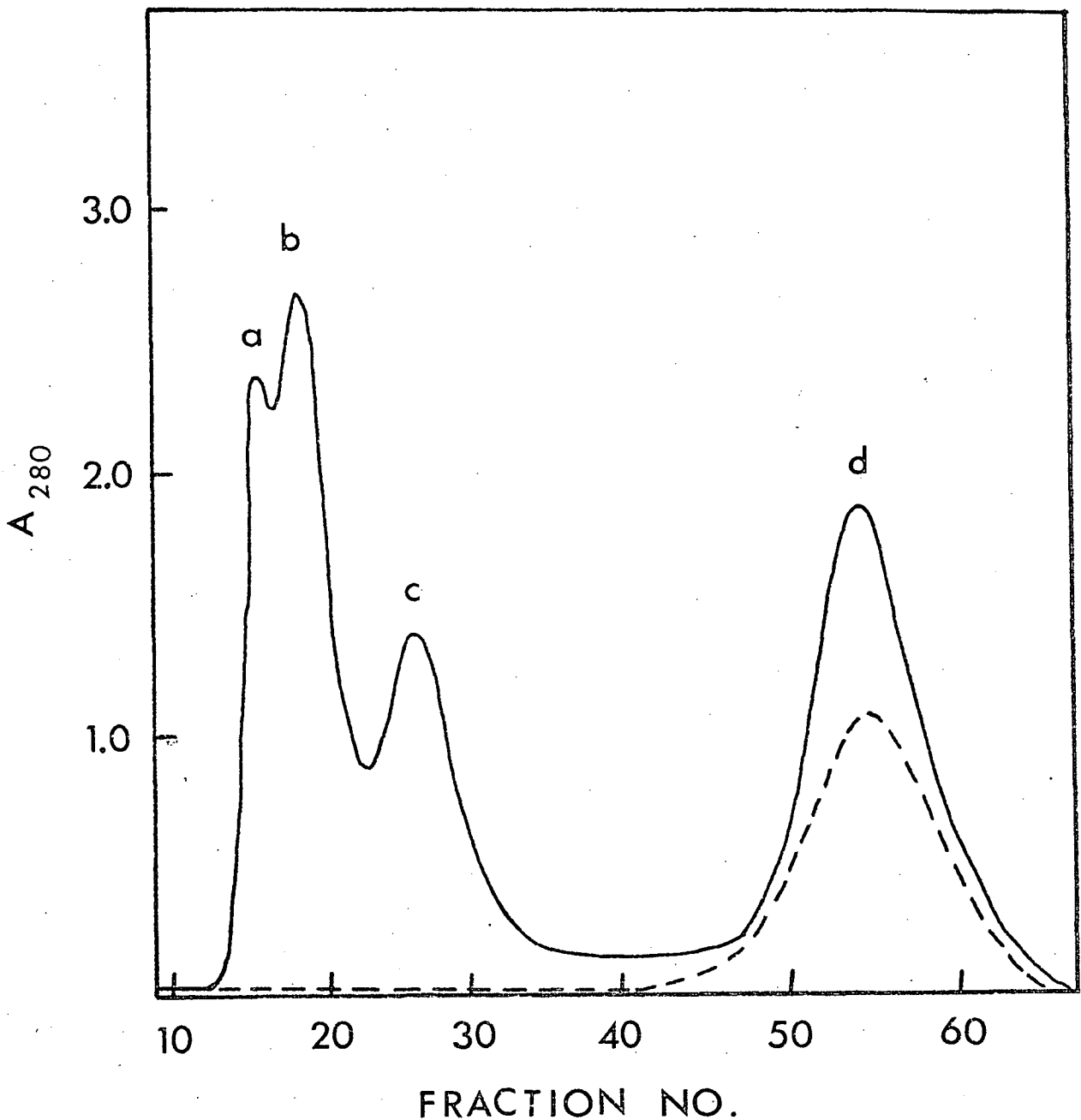
Figure 6. Sephadex G-50 gel filtration of whey (____) and ultrafiltered whey (----). Conditions: gel bed size 20 X 33 cm, flow rate 85 ml/hr, eluting buffer 0.05 M NH_4OA_c, pH 4.5, column operated in an upward flow manner. a. casein fraction; b. β-lactoglobulin; c. α-lactalbumin; d. low molecular weight nitrogen containing material.
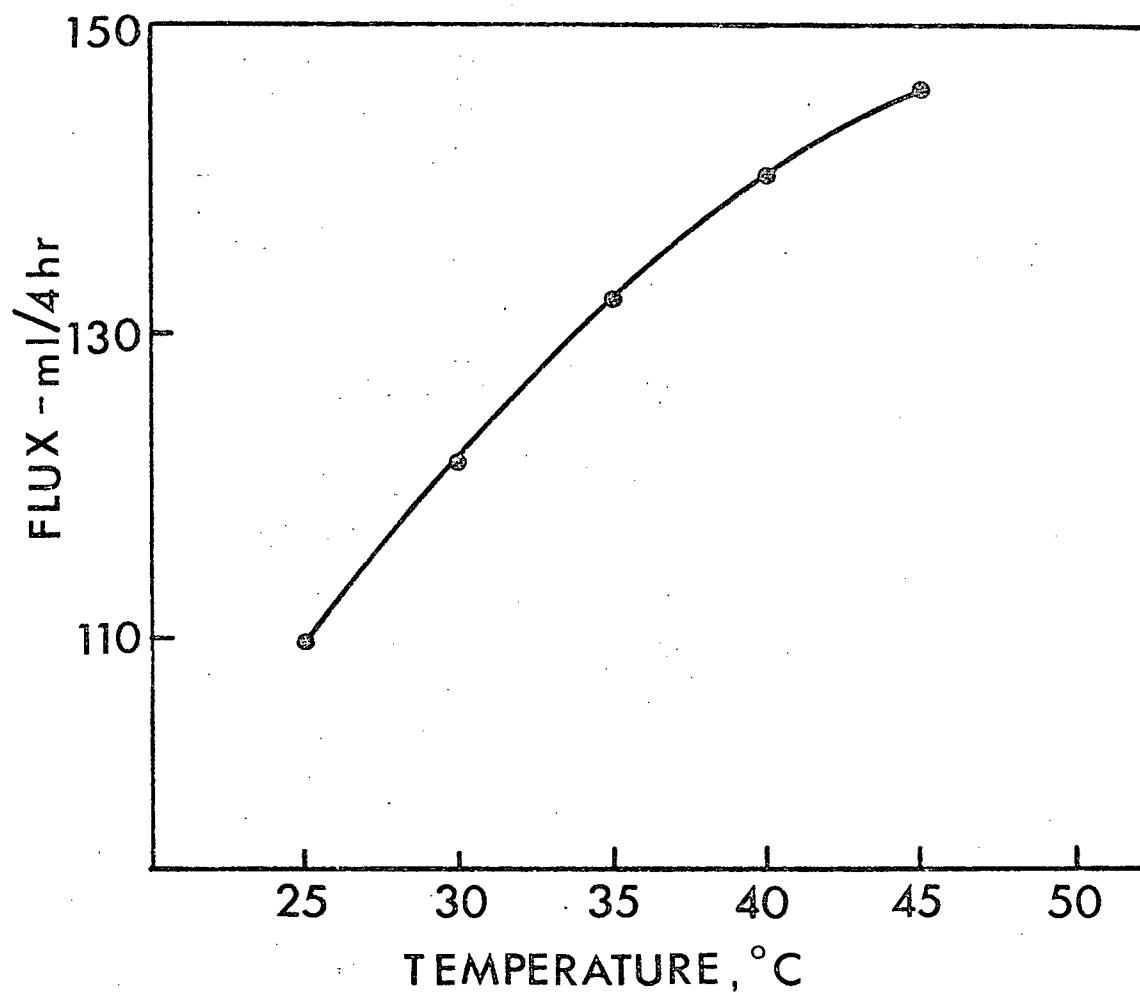
Figure 7.   Effect of temperature on flux rate

(Figure 8). Continuous washing was the most efficient method
to increase the protein concentration.

pH Adjustment of Wash Water

The pH adjustment of the wash water is very critical
in optimizing permeate flux and extending membrane life. Pre-
liminary studies (Figure 9) indicate that the pH of the water
adjusted to the basic side affords best results.
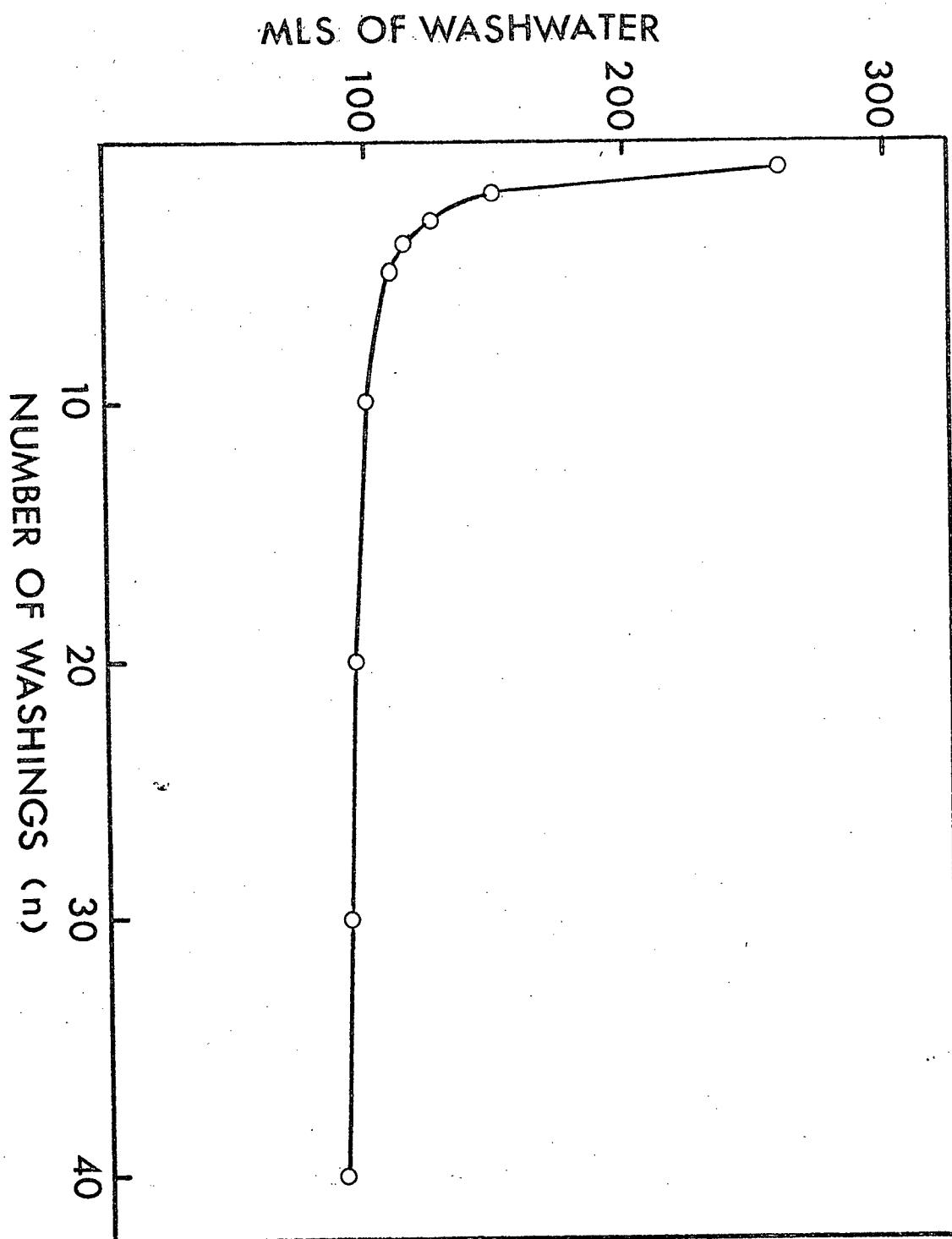
Figure 8. Graph showing washing conditions necessary to increase protein concentration to greater than 80%.
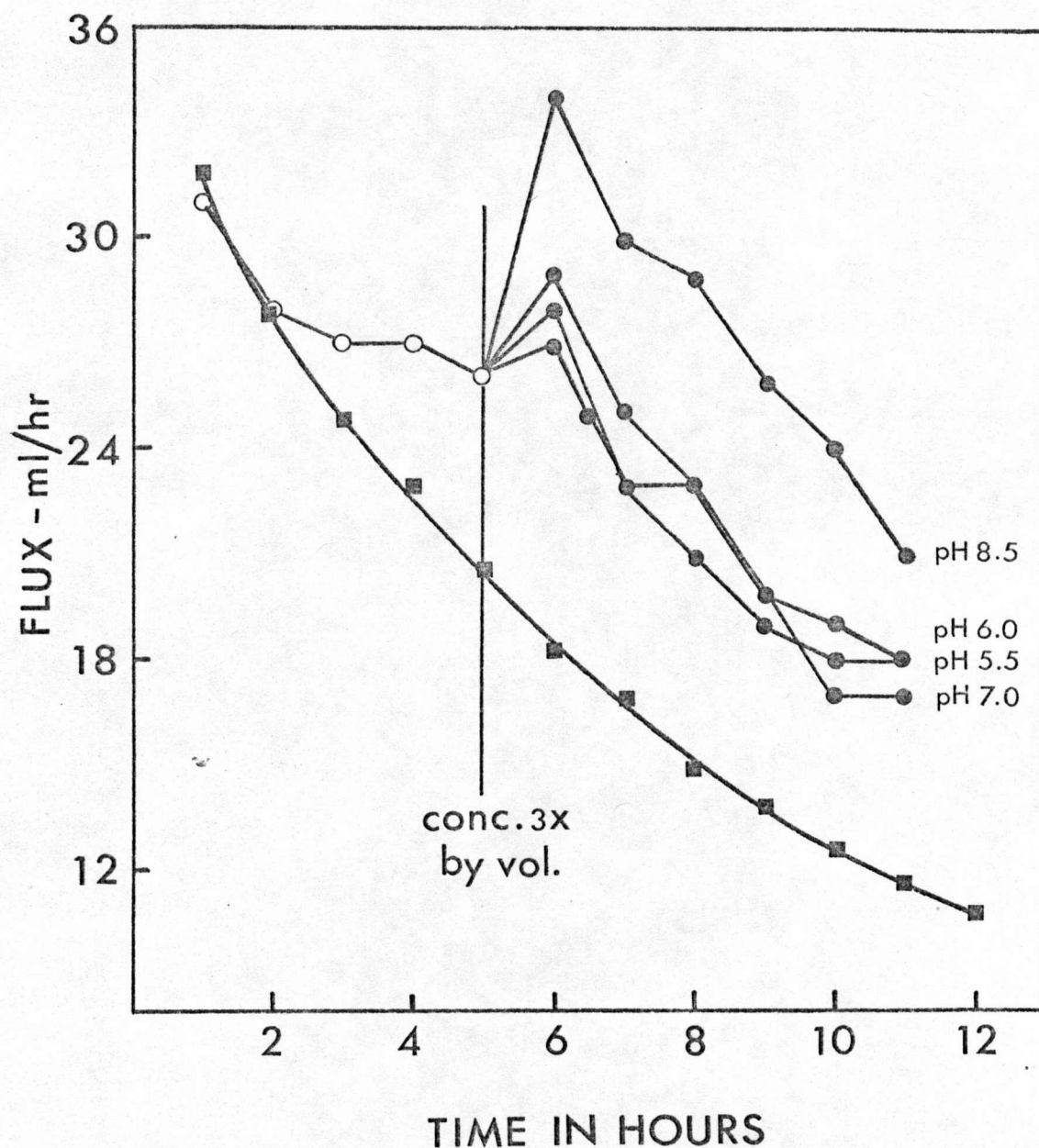
Figure 9. The effect of pH adjustment of the wash water on permeate flux.

■—■ Whey pasteurized at 72°C for 10 min and left at pH 4.6.

○—○ Whey pasteurized at 72°C for 10 min, adjusted to pH 7.0 and centrifuged at 1,500 X g for 5 min at 0°C the supernatant of which was used for ultrafiltration.

●—● pH adjusted wash water as indicated.

DISCUSSION

Concentration and fractionation of whey by ultra-filtration has been studied extensively (13, 32, 34, 35, 36, 37, 38), yet few workers have sought optimum conditions for the process. The retentive properties of various membranes under different conditions were investigated to determine maximum flux rates with minimum fouling.

Data summarized in Tables I and II show that permeation rates varied with each membrane. Since optimum protein retention was desired with maximum flux a PM 30 membrane with molecular cutoff at 30,000 was chosen. Although lactose retention is high, 100% removal of lactose from whey could be achieved only by removing 100% of the water. Data in Table II show a lactose retention of 52.6% for PM 30 at 48% volume reduction. Whereas, with a 80% volume reduction, 87% of the lactose was removed. Therefore, the protein-to-lactose ratio is directly related to the permeate removed.

Figure 1 shows that the permeation rate of whey decreased with time. This is a direct result of the phenomenon, concentration polarization which is the accumulation of rejected dissolved solutes at the membrane surface. The accumulation of these at the boundary rapidly results in the formation of a viscous concentrated or gelatinous layer which offers resistance to flow. Concurrent with this is the increase in osmotic pressure opposing the process as the

concentration increases. The components mainly responsible are protein, lactose and the salts. Fouling of a third type termed pore plugging also occurs and unlike the formation of the gel-like layer occurs more rapidly (47). This is so because of the early predominance of pore flow and the limited opportunity for convection flow to remove macrosolutes or colloidal particles that become lodged in the pores. This results in a decrease in permeation rate. We found that this problem could be partially solved by centrifuging (clarifying) whey samples prior to ultrafiltration such that colloidal particles would be precipitated (Figure 3).

Carrying out the process at a high rate of shear resulted in greater permeation rates. Yet, as pointed out by Forbes (14) high shearing rates carry an energy penalty and any system optimization requires compromise between flux improvement and energy expenditure. It was found that operating at a pressure of 60 psi satisfied both conditions.

Data reported in Figure 1 are similar to that obtained by other workers where optimum conditions for the process were not investigated. Figure 2 shows that pH adjustment of the whey accompanied by clarification greatly increases the permeate flux. The minimum is associated with pH 6.0 due to the precipitation of denatured proteins and/ or calcium and magnesium salts not removed by centrifugation prior to ultrafiltration. Maximum permeate flux is at pH 7.0.

This arises since on the alkali side the protein denatures and after centrifuging is precipitated. Minimum flux is between pH 3 - 5 since this is the isoelectric point of the protein, thus aggregation occurs tending to give lower diffusion rates.

Centrifuging at pH 7.0 results in a decrease of protein in the supernatant (Figure 3) and partially explains the increase in flux rate if protein is assumed to be responsible for membrane blockage (28). Experimentation has shown that if whey is ultrafiltered at pH 4.6 at a protein concentration consistent with the centrifuged sample at pH 7.0 the flux rate is considerably lower. Therefore, pH adjustment followed by centrifugation is very critical in optimizing the process. Centrifuging at neutral pH removes macrosolutes or colloidal particles which would otherwise become lodged in the membrane pores and decrease the flux rate through fouling.

Lim et al. (28) on the basis of estimation of the molecular weight together with electrophoretic patterns showed that the major components responsible for clogging were: 1) Casein; 2) β-lactoglobulin; 3) α-lactalbumin, and 4) a nonprotein fraction. Polyacrylamide gel electrophoresis (Figure 4) shows that only β-casein and α_s-casein are responsible for blockage. The protein forms a gel which adheres to the membrane and in effect adds another filtering layer. Further analysis of the precipitate suggested that

Ca and Mg salts as well as lactose may also be responsible,
but to a much smaller degree.

Gel filtration (Figure 6) indicated that by using
a PM30 membrane all the proteins are retained while low
molecular weight nitrogen-containing materials pass through.
These results are consistent with those in Table II where
protein retention was 94.4%. Moreover, peaks b and c
(Figure 6) represented by β-lactoglobulin and αlactalbumin
respectively (21) appeared in the gel electrophoresis studies
(Figure 4) as did peak a which is assumed to be residual
casein. This latter fraction was probably not affected by
rennet and or bacterial action during the cheese making
process or possibly may be casein fines from the cottage
cheese itself. Although Figure 6 indicates that all the low
molecular weight nitrogen containing material passes through,
some remains behind in the concentrate. These fractions
tend to penetrate and become lodged in the membrane and
therefore decrease flow rate through pore plugging.

Generally, increasing pressure does not appear to
increase flux significantly (19, 20). All that happens is
a thickening of the boundary layer until the rate of material
arrival equals the rate of back-diffusion (14,16). Operating
at 60 psi was used as a means of creating shear in the
passage of whey through the membrane and thereby increasing
flow rate. Although identical flux rate was obtained at

lower pressures if turbulence by means of the magnetic stirrer
was increased, it was found that by operating at a constant
pressure of 60 psi and maintaining turbulence at a constant
speed, resulted in satisfactory flow rates.

Operating at high temperatures increases permeate
flux (Figure 7) due to viscosity decrease. As pointed out
by Roualeyn et al. (54) operating at the highest possible
temperature compatible with the membrane gives best results.
They found that every 20° rise in temperature is accompanied
by an increase in permeate flux of approximately 40%, consistent
with our results. Moreover, operating at elevated temperatures
above 50°C was found to decrease microbiological activity (54).

Kissinger et al. (25) showed that by inoculating the
membranes with a mixed bacterial culture followed by incubation
had no destructive effect yet severe sliming of the membrane
occurred. In our work microbial growth was not a problem
since all samples were pasteurized before ultrafiltration.

Although standard plate counts were not done it was
assumed that bacterial growth was minimum since after 20 hours
of ultrafiltering the pH of the whey remained the same.
Generally the optimum temperature appears to be around 50°C
for maximum flux (20). This temperature has additional
advantages from the standpoint of plant operation since the
whey comes off the cheese vats about this temperature.

Only recently has washing of the concentrate been

implemented to increase its protein concentration (47). Protein values of about 80% on a dry weight basis were obtained by Peri et al. (51) but their washing technique involved four purification steps. As pointed out by these same workers this so-called diafiltration step may be started from the beginning of the operation or after a preliminary step of simple ultrafiltration. The correct combination of ultrafiltration and diafiltration is the key factor in defining the optimum process.

Our theoretical calculations (Figure 8) indicated that continuous washing would yield high protein concentrates while minimizing water consumption. According to the data reported in Table III the optimum process for obtaining protein concentrates with greater than 70% protein on a dry weight basis as well as being low in ash would be accomplished by continuous washing.

The retention of calcium is high because this fraction is associated with the proteins or is present as colloidal calcium phospahte. It is evident therefore that the fraction of calcium that can be permeate decreases during the process as protein concentration increases. Moreover, pH adjustment of the wash water (Figure 9) toward the basic side increases flux rate since the protein remains denatured and aggregation cannot occur. It therefore becomes economically possible to obtain a product with a high protein-to-lactose ratio. If membranes can be improved to permit 0% retention

of lactose rather than 18% reported here, concentrates with significantly higher protein could be produced.

Peri et al. (51) showed that diafiltration of skim milk increased permeation rate whereas those for whey decreased. Data reported in Figure 9 also showed a decrease in permeation rates during the diafiltration process. The explanation of this is that microbial and enzymatic degradation of proteins results during the cheese making process. This tends to increase the proportion of non-protein nitrogen and of proteoses and peptones in the whey. These fractions have dimensions similar to those of the membrane pores and therefore tend to penetrate into the membrane structure and plug it.

Results showed that the BOD of whey can be reduced in the order of 20%. Although this is not a substantial decrease from a pollution standpoint, it is possible to couple ultrafiltration with reverse osmosis and thereby reduce the BOD by 99% (20, 21). Moreover, the use of sanitary RO equipment makes it possible to control the microbiological quality of water. Thus, reuse of the curd rinsing water in cottage cheese manufacture would be possible according to the scheme similar to that illustrated in Figure 10. Combination of the two processes of ultrafiltration and reverse osmosis is now under investigation in this lab.

High protein concentrates are attractive from the

Curd
Skim ⟶ Cheese ⟶ 1st Wash ⟶ 2nd Wash ⟶ 3rd Wash ⟶ Curd

Whey
-Adjust pH
  to 7.0
-Pasteurize
  72°C for
  10 min.

Separator 1500Xg
      for 5
      min.

Separator 1500Xg
      for 5
      min.

Dilute   Sediment   Water
Whey
-Adjust pH
  to 8.5

Sediment   Whey ⟶ Ultrafiltration   Reverse Osmosis

                                                   Lactose

                                        UF Permeate
                                         - lactose
                                         - water
                                         - minerals
                                         - salts

Protein Concentrate

Spray Dry   Spray Dry
   or   or
use as a concentrated   Enzyme treatment
solution.

73% protein on a dry   86% lactose on a
        basis   dry basis

Figure 10. Flow diagram for the preparation of whey, protein,
            crude soluble lactose and the reuse of curd
            rinsing water.

standpoint of increased utilization of whey (18), but a
pollution problem involving disposal of the permeate remains.
The lactose recovered in the permeate is greater than 85%,
easily soluble in water and therefore has possible applica-
tions in the food area (5, 19).

As specified earlier in this paper the data
reported refer to cheese whey and a 1250 mm^2 membrane area.
The time would be proportionally reduced or the capacity
proportionally increased by an increase of membrane area.

Optimum conditions suggested in this work are
relative to our experimental conditions and apparatus and
are fundamentally dependent on membrane type, temperature,
pH and flow conditions.

PART II   PREPARATION OF FERRIC WHEY

PROTEIN BY HEATING

ABSTRACT

Soluble ferric whey protein containing more than 80% protein was prepared by an improved heating method at acid pH. Whey was concentrated 3 to 4 times and heated to $92^{\circ}C$ for 15 min after adding $FeCl_3$ and adjusting to pH 2.5. After centrifugation, the sediment was washed with dilute HCl at pH 2.5, dissolved and dried. Lactose was collected from the supernatant.

Preconcentration of whey before heating increased the yield of ferric protein to 60% of total N. The optimum pH for obtaining soluble products with the highest yield was 2.5 to 3.5 depending on the amount of $FeCl_3$ added. Washing the precipitated protein at pH 2.5 to remove excess salts and high speed blending to dissolve washed precipitates at neutral pH were necessary for obtaining high solubility of the dried protein.

Iron in ferric whey protein was available to growing chicks. Results showed no significant difference in availability between ferric whey protein and the $FeSO_4$ standard.

INTRODUCTION

Since the success in manufacturing ferrilactin from cheese whey by Block et al. (6) in 1953, several modifications (22, 23) have been submitted for separation of whey proteins with iron salts.

In the ferrilactin manufacturing, $FeCl_3$ is added to whey and the pH adjusted after which it is frozen to crystallize the protein and then filtered. However, this procedure especially freezing and filtration, is lengthy and tedious. Imado et al. (22) utilized whole whey without separating ferrilactin after addition of $FeCl_3$. They added polyphosphates to prevent brown colour due to ferric iron in ion-exchanged ferric whey. The product was successfully used to fortify fluid milk, dry milk and dry baby formula without causing vitamin destruction or flavor deterioration during storage.

Jones et al. (23) developed a method to separate whey protein utilizing a ferripolyphosphate. Although their cold process is a distinct advantage, the protein content in the dry products was relatively low with a considerably higher phosphate content that may disturb normal kidney function especially in babies.

We have been trying to establish methods to separate ferric whey proteins for fortification of iron to food products. Emphasis was placed on higher yield and solubility of the dry products. The acid washing method of Harwalker and Emmons (17)was effectively employed to

maintain high solubility for whey proteins separated by heating.
A study on cold processes using ultrafiltration for unde-
natured ferric whey protein is described in Part I.

This paper reports the results of the preparation of
ferric whey protein with a protein content greater than 80%.
The conventional heating method at an acid pH was modified
to obtain soluble products with high yields.

MATERIALS AND METHODS

Preparation of Ferric Whey

Whey was obtained from a local dairy (Fraser Valley Milk Producers Association, Vancouver, Canada) and clarified by centrifuging at 1,500 X g for 10 min at $0^{\circ}C$ in a Sorvall RC2-B centrifuge. The supernatant was concentrated three times by volume on a Flash Evaporator (Buckler Instruments, Fort Lee, New Jersey) held at $50^{\circ}C$.

Reverse Osmosis

Reverse Osmosis experiments were conducted on a larger assembly made by Fre-Del Engineering Corporation, Santa Ana, California, U.S.A. The membrane unit is of a sandwich construction which directs the flow of liquid between two cellulose acetate membranes 25 $inch^2$ in area, supported by stainless steel sintered metal back up plates. As whey flowed through the membranes under a pressure of 1000 psi, water passed out of the hose bibs at the base of the membrane unit and the concentrate was re-circulated. Pressure was maintained constant by the aid of a surge suppressor and an adjustable back pressure control valve. Volumes of 1500 ml were used. The membrane was specified as KP96 type with salt rejection at 95%. All experiments were conducted at temperatures not exceeding $25^{\circ}C$.

Iron Depletion

The method of Pla and Frits (53) was followed using one-day-old cockerels. The chicks were housed in galvanized

cages. The basal diet and de-ionized water were supplied ad libitum. Water troughs were painted with a non leaded paint so that rusting would be inhibited. After two weeks on the basal diet hemoglobin and hematocrit were determined by the cyanmethemoglobin method of Crosby et al. (11) and by a microcapillary centrifuge method (10),respectively. Hemoglobin was determined using Cyanmethemoglobin Standards and Cyanmethemoglobin Reagent (Hycel, Inc., Houston, Texas). Blood samples were drawn from the wing vein and also by heart puncture. Both techniques gave similar results. After chicks were satisfactorily depleted (hemoglobin < 5 gm/100 ml and/or hematocrit < 24%) they were divided into three groups of ten. Groups I, II and III received diets containing 20 mg Fe/kg in the forms of standard $FeSO_4$, ferric whey protein prepared by the heating method described in this paper, and undenatured whey protein, i.e. dried cottage cheese whey containing 224 ug Fe/ml whey respectively. After 2 weeks on the test diets and each subsequent week thereafter for 4 weeks hemoglobin and hematocrit analyses were done. The responses were compared to the $FeSO_4$ standard by analysis of variance and Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The outline of the procedure finally adopted is shown in Figure 11.

Effect of Preconcentration

To increase the yield, whey was concentrated 3 times and heated to 80, 90 or $100^{O}C$ for 10 min at pH 4.5. Protein was analyzed in the supernatant after centrifuging at 2,000 X g for 10 min. The control was boiled for 15 min without preconcentration. The protein sedimented was 44, 56 and 61% by heating preconcentrated whey to 80, 90 and $100^{O}C$ compared to 52% for the control. The higher yield can be expected by preconcentration under the same heating conditions. However, preconcentrating greater than 4 times decreased the yield due to excessive viscosity which prevents sedimentation of coagulated proteins.

Effect of pH

The pH was varied from 4.5 to 2.5 for concentrated wheys, which were subsequently heated to $85^{O}C$ for 10 min (Table IV). On average the highest yield was obtained at pH 2.5. However, at this pH the solubility of the whey with low iron content was markedly depressed. The pH optimum for obtaining soluble products with high yields, were 2.5 and 3.5 with and without iron respectively.

Effect of Washing

To decrease ash content and improve solubility, the

Cheese Whey, 2 kg

Evaporate 3 to 4 times

Concentrate (500 to 650 g)

Add .21 ml of 30% $FeCl_3$[1], adjust

to pH 2.5, heat to 92°C for 15 min

and centrifuge

Sediment.

   Wash with dilute HCl

   at pH 2.5. The 1st wash ──→ Supernatant ←──────────

Washed precipitate

   Dissolve in dilute

   NaOH at pH 7, blend

   or homogenize and

   spray dry

Supernatant

   Evaporate, cool and

   centrifuge

Sediment → Supernatant

   Dissolve at

   pH 7 and

   centrifuge

Sediment

(Discard)

Supernatant

   Spray dry

Protein powder

   14 g

   60% of total N in the

   starting whey

Crude soluble

lactose[2]

   65 g

[1] The retention of iron in the final product was approximately 70%. Total protein in 2 kg of whey, appr. 18 g; 60% yield, appr. 11 g. .21 ml of 30% $FeCl_3$ contains $.3 \times 55.85/162.2 \times .21 = .0216$ g; the retention 70%, appr. 15 mg Fe. Fe in protein in final product, appr. .145.

Figure 11. Flow diagram of preparation of ferric whey protein and crude soluble lactose.

Table IV. Effect of pH on the solubility and the recovery

of protein in final product.

pH	Fe	Solubility	Protein Recovery
	µg/ml whey	%	%
4.5	224	99.5	42.4
	56	100	36.7
	10.8	98	33.9
	0	100	28.2
3.5	224	100	55.7
	56	100	40.4
	10.8	98	39.3
	0	100	35.7
2.5	224	99	60.3
	56	100	55.3
	10.8	64	57.9
	0	70.3	57.5

Concentrated wheys were heated at $85^{O}C$ for 10 min and the

protein precipitates were washed twice.

washing method of Harwalker and Emmons (17) was employed except that the pH was adjusted to 2.5 instead of 3.5. The refracto-metric observation of solids in the wash water indicated that washing 2 to 3 times was usually adequate to eliminate most of lactose and salts. Protein in the total solid was increased from 40% to 75, 80, and 90 and even 95% by washing one, two and three times respectively.

It was also found that blending washed protein precipitates at high speeds or homogenizing to disintegrate coagulated proteins when dissolving at neutral pH was important for obtaining soluble dry products.

Comparison with a conventional heating method

The combination of selected conditions (preconcentration, pH, temperature and washing) was applied and compared to a control prepared by the conventional heating method under an acid condition. The pH during washing was maintained at 2.5 except for the control, for which plain water was used. The results are shown in Table V. Satisfactory protein recovery and solubility were obtained by heating cottage cheese whey without iron to $92^{O}C$ for 10 min at pH 3.5. For whey with iron at the level of 2.9% in protein (224 µg/ml whey), heating to $92^{O}C$ at pH 2.5 did not decrease the solubility or the protein content in the dry product. Temperatures higher than $92^{O}C$ increased gelatinous sediments which decreased the solubility after drying. The quality of the control product was poor with low protein recovery and solubility. The protein recovery for the conventional method was considerably lower than the preconcentration control (52%) probably resulting

Table V.   Comparison of heating methods for preparation of
           soluble whey protein powder.

Whey	pH	Temper-ature	Fe	Protein in dry weight	Protein recovery	Solubility
		C	µg/ml whey	%	%	%
Unconcen-trated	4.5	98	0	76.4	15.6	25.9
Concen-trated	3.5	85	0	84.4	39.2	96.7
4 times	3.5	92	0	90.2	61.0	92.5
	2.5	85	224	60.0	60.3	99.0
	2.5	92	224	84.2	60.1	91.2

Heated for 10 min.

from a loss of protein during washing with plain water. As seen in Tables V and VI, the dry products contained more than 80% protein with solubilities greater than 90% in water at 25°C. The procedure was equally well applied to both cottage cheese whey and cheddar cheese whey.

A good yield of crude lactose that was easily soluble in water was obtained from the supernatant after sedimentation of the proteins (Figure 10 and Table VI).

Gel electrophoresis

Block et al. (6) showed by paper electrophoresis of ferrilactin that both β-lactoglobulin and α-lactalbumin migrated while one spot remained at the origin. Jones et al. (23) using both urea and nonurea gels found that the addition of ferripolyphosphate to whey caused precipitation of β-lactoglobulin and α-lactalbumin. Ferric whey protein prepared by our method exhibited bands similar to those of whole whey, implying the precipitation of both β-lactoglobulin and α-lactalbumin by heating with $FeCl_3$ (Figure 12). Undenatured ferric whey protein, separated by ultrafiltration, showed the same result.

Reverse Osmosis

Treatment of reconstituted ferric whey supernatant increased flux rate greater than 2X as compared to conventional cheese whey. This increase is attributed to the complete removal of whey proteins as shown by gel filtration studies.

Table VI. Analysis of whey protein powder and crude soluble

lactose (freeze dried).

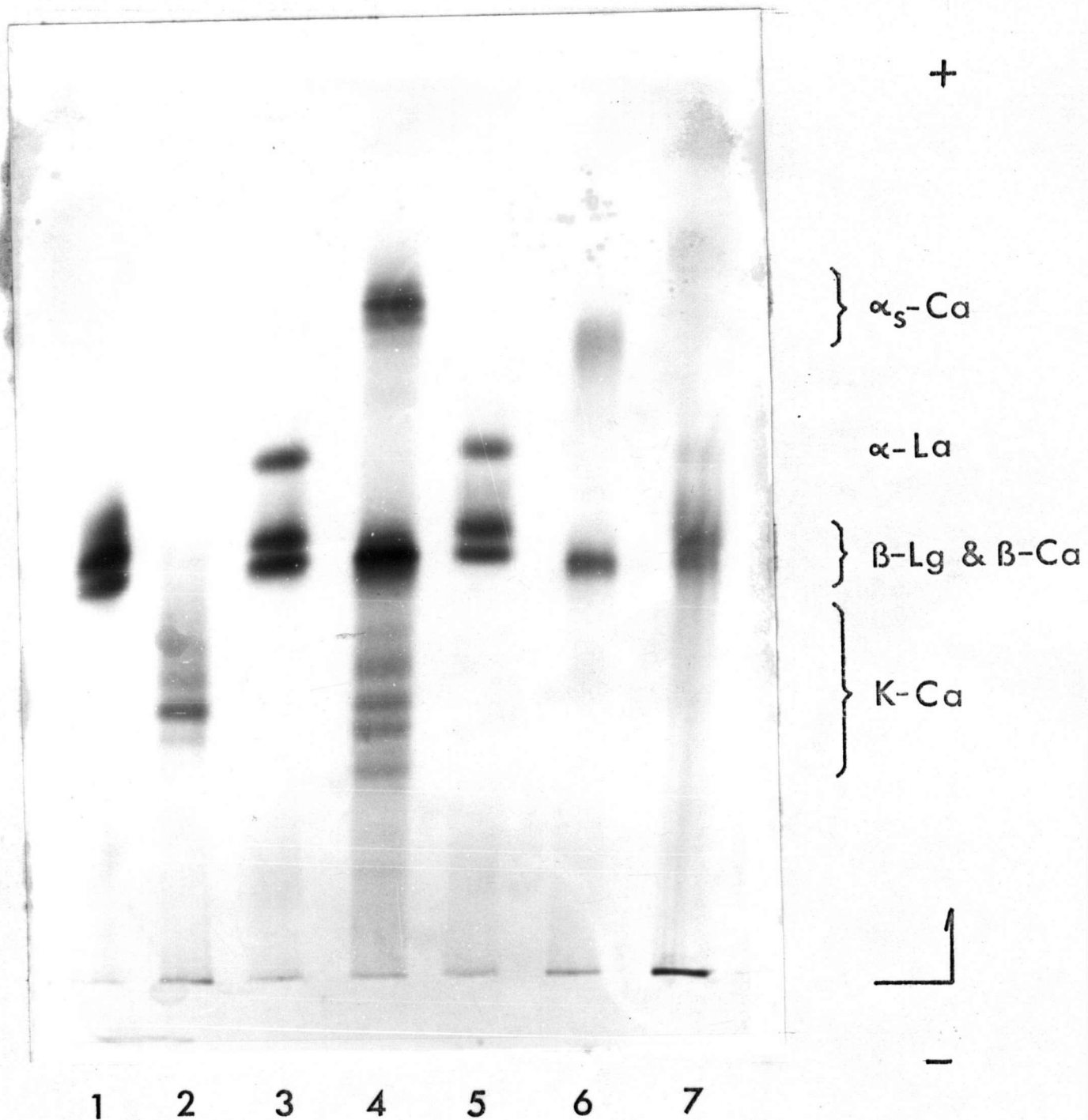| Product	Moisture	Protein	Lactose	Ash	Fe[1]
	%	%	%	%	%
Cottage cheese whey protein	4.1	87.6	1.7	7.0	.3
Cheddar cheese whey protein	3.9	81.0	4.0	4.2	.18
1st lactose	1.7	4.4	84.3	4.5	
2nd lactose	4.9	4.4	87.0	7.5	

[1] Adjustable depending on purpose.

Figure 12. Polyacrylamide gel electrophoresis of whey
proteins. 1. β-lactoglobulin; 2. K-casein;
3. whey pasteurized at 72 C for 10 min after
adjusting the pH 7.0; 4. acid casein;
5. the supernatant after centrifuging No.3
at 1,500 X g for 10 min at 0 C; 6. the
sediment of No. 5; 7. ferric whey protein.

Unlike UF, pH adjustment to 7.0 followed by centrifugation decreased rather than increased permeate flux. This is assumed to be due to the formation of insoluble colloidal particles which become lodged in the membrane pores or accumulate on the surface. In UF these collodial particles are small enough to pass through and protein becomes the major cause of blockage.

Calcium was removed from the ferric whey supernatant by potassium oxalate and RO treatment substantially increased flux rate as compared to the untreated ferric whey. This clearly implicates the involvement of salts in the blockage of RO membranes and further studies are presently underway to elucidate these complex situations.

Available Iron

Block et al. (6) found that the iron of ferrilactin was readily available to aenemic rats for regeneration of hemoglobin. Imado et al.(22) reported that there was no difference in the hemoglobin value and the serum iron among rats fed with $FeSO_4$, ferric citrate and their ferric whey products. However, the iron deposits especially the hemosiderin iron were higher for the groups with whey, sulfate, and whey in this order. Because of frequent disagreement among researchers of feeding test results, analytical procedures for available iron have been under intensive re-examination.. Recently, a more reliable method was submitted

(1, 53) and the availability of iron in our ferric whey protein
was estimated by this new procedure (Table VII). Iron present
in ferric proteins both denatured and undenatured was readily
available to chicks and there was no significant difference
from available iron in the $FeSO_4$ standard.

Table VII.   Mean terminal values and standard deviations for
             hemoglobin, hematocrit and liver iron of chicks
             fed different sources of iron.

Iron Source	Iron dose	Hemoglobin	Hematocrit	Liver iron
	-mg/kg diet-	-g/100 ml-	-%-	-μg/g wet wt-
Basal diet	0	7.69 ± 1.49^a	28.24 ± 5.27^a	
FeSO$_4$	20	9.72 ± 1.18^b	41.76 ± 4.22^b	84.5 ± 12.5^a
Denatured ferric whey proteins	20	$9.76 \pm .59^b$	41.25 ± 1.49^b	63.0 ± 17.5^a
Undenatured ferric whey Proteins	20	10.89 ± 1.23^c	40.63 ± 2.95^b	111.9 ± 39.2^a

'a', 'b' and 'c' are significantly different by Duncan's
multiple range test ($P < .05$)

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APPENDIX

TABLE I

Analysis of variance in hemoglobin after repletion:

Source	d.f.	S.S.	M.S.	F.
diets	3	53.213	17.738	14.964
error	36	42.672	1.185	
total	39	95.885		

Duncan's Multiple Range Test (P < .05)

A   B_____C   D

TABLE II

Analysis of variance in hematocrit after repletion:

Source	d.f.	S.S.	M.S.	F.
diets	3	1264.320	421.440	26.946
error	36	563.039	15.639	
total	39	1827.359		

Duncan's Multiple Range Test (P < .05)

A   B_____C_____D

TABLE III

Analysis of variance of iron in liver samples after repletion

Source	d.f.	S.S.	M.S.	F.
diets	2	.00239	.00119	.8959
error	3	.00400	.00134	
total	4	.00640		

Duncan's Multiple Range Test (P < .05)

No significant difference